THE CORRELATION OF DESOXYRIBONUCLEIC ACID SYNTHESIS AND THE RATE OF RESPIRATION IN THE SEA URCHIN EMBRYO[1]

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Measurements of the rate of respiration of sea urchin eggs from fertilization through early development have been reported by Warburg (1915), Gray (1925, 1926), Rapkine (1927), Ephrussi (1933), Tyler (1936b), Lindahl (1939), Horowitz (1940), Hutchens *et al.* (1942), Borei (1948), and others. In all experiments the rate of oxygen uptake is shown to increase during development. A number of attempts have been made in the past to learn about factors controlling, or related to, this increase. It seems not to be related closely to the increase in total nuclear volume (Godlewski, 1908), the increase in the number of cells of the embryo (Warburg, 1915; Tyler, 1936a), nor to the increase in the rate of cleavage of the cells of the embryo (Gray, 1926; Tyler, 1936b).

An experiment that suggests a possible relation between the rate of respiration and the content of desoxyribonucleic acid (DNA) was done by Brachet (1938). The oxygen consumption of fertilized eggs of *Chaetopterus* as well as eggs activated by KCl was measured and the respiration of the KCl-activated eggs, which differentiate without cleavage, was found to increase at a much slower rate than that of the fertilized eggs. It was found that the larvae from the KCl-activated eggs contained only 30% of the DNA present in the fertilized eggs several hours after the activation or fertilization. Brachet's interpretation was that (p. 97) "both the reduced oxygen uptake and the slower development are linked together; such a conclusion could support Tyler's opinion that part of the energy available in the egg is needed for the growth and differentiation processes taking place during development." Similarly, Tyler and Horowitz (1938) concluded, from an analysis of the effects of phenylurethane on respiratory rates of *Urechis* embryos, that the normal rise in rate of respiration is probably connected with nuclear division.

The purpose of the work recorded here has been to determine whether a correlation does exist between the rate of oxygen consumption and the DNA content of sea urchin embryos. If such a relationship were found, another conclusion, alternative to Brachet's, could be drawn: it would be possible that DNA, as a constituent of the genome of the cell, is limiting the production or activation of respiratory enzymes which would be responsible for the rate of oxygen consumption.

This problem was undertaken at the suggestion of Dr. Albert Tyler, who had calculated from published figures that a close relation between these two events was

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likely. The authors are indebted to Dr. Tyler for proposing the investigation and for critically reading the manuscript.

Materials and Methods

The eggs of *Strongylocentrotus purpuratus* (Stimpson), collected at San Juan Island, Washington, were used in this study. For each experiment the eggs of only one sea urchin were used. To induce spawning of both sexes the urchins were injected with 4.25% KCl (Tyler, 1949). The eggs were washed with filtered sea water at least three times or until 95 to 100% fertilization of the eggs was attained. If near 100% fertilization were not possible, or if the eggs were abnormal in any respect, they were not used.

From a 5% suspension of eggs, 5- or 10-ml. aliquots were taken for egg counts and for the determination of oxygen consumption and DNA content of the unfertilized eggs. After dilution of the remaining egg suspension the eggs were fertilized, washed free of excess sperm, and the original egg concentration re-established. Aliquots of approximately 10 ml. were then taken for determinations at the two-cell stage; for all later stages 10-ml. aliquots were added to 200 ml. of filtered sea water for the first experiment and to 500 ml. for the second experiment. The embryos were cultured in a constant temperature bath at 18° C. If development did not proceed normally with the survival of more than 85% of the embryos, the results were not used.

For the determination of the rate of oxygen consumption and quantity of DNA, the embryos were harvested by centrifuging. For the swimming stages the centrifuging was done with a modified Foerst plankton centrifuge constructed so that the embryos were in contact with glass or Plexiglas only. The embryos were subjected to a maximal RCF of 150 × g for 15 to 20 minutes. They showed no visible evidence of injury following this treatment.

The number of eggs was used as a parameter for the measurement of the DNA content and the rate of oxygen consumption. Egg counts were made on an aliquot of the unfertilized eggs which was diluted until there were from 100 to 200 eggs per 10 ml. Five to 10 counts were made of 10-ml. aliquots of this dilution. The aliquots were transferred to a narrow, shallow plastic trough where the eggs settled in a row and could be counted readily at low magnification. The variation in the egg counts was less than 10%.[2] This need only be considered when comparing the first experiment with the second where different egg batches were used.

As soon as possible after harvesting, the respiration of the embryos was measured with the Warburg constant volume respirometer. The concentration of eggs used varied from 5 to 10% depending on the stage of development of the embryos. The speed of shaking used was 120 cycles per minute with an amplitude of 2.5 centimeters. These conditions of crowding and shaking were not harmful to the embryos, as determined by examination after respiration measurements. The temperature at which the measurements were made was 18 ± 0.01° C.

After the rate of respiration had been determined over a period of from ½ to 2

[2] In Experiment 1, the average number of eggs is 56,000 per ml., and the 95% confidence interval is 55,300 to 56,600. In Experiment 2, the average number of eggs is 51,800 per milliliter with a 95% confidence interval of 49,400 to 54,100.

hours, the embryos were removed quantitatively from the Warburg flasks, centrifuged mildly, and the excess sea water removed. The packed embryos were then frozen at $-35°$ C. until they were analyzed for DNA content. The period of frozen storage was about two weeks.

The analyses for DNA were based on determining the amount of DNA phosphorus and were made by a combination of parts of the methods of Schmidt and Thannhauser (1945) and Schneider (1945) with the modification for the direct analysis of DNA by Schmidt, Hecht and Thannhauser (1948). The separated DNA was analyzed for total phosphorus content by the method of Berenblum and Chain (1938) following perchloric acid digestion.

Abrams (1951) reports that re-precipitation of the DNA is necessary for the removal of all of the contaminating RNA. To determine if this were necessary for the material used in the present investigation, DNA determinations were made on two aliquots of unfertilized eggs without re-precipitation of the DNA and on two aliquots with re-precipitation of the DNA two times. This is a severe test of the

TABLE I

The effect of re-precipitation of DNA on its quantitative determination

Unfertilized egg samples	Micrograms DNA P/10^6 eggs	Average
Washed twice	0.932 0.988	0.960
Precipitated twice	0.859 0.942	0.901

method because the ratio of RNA to DNA is very high in the unfertilized egg. The results are given in Table I.

The difference in DNA phosphorus obtained by the two methods may be considered negligible. Nevertheless, in the second experiment all of the samples of DNA were re-precipitated once and washed once.

RESULTS

To determine whether a correlation exists between the rate of respiration and the content of DNA phosphorus in the normal development of the sea urchin embryo, these were measured at intervals between fertilization and the early pluteus stage. The first series, Experiment 1, was carried out in February, 1951, and the second, Experiment 2, in August, 1951. Each series consists essentially of two parallel experiments in that both the respiration and DNA phosphorus determinations were made on duplicate aliquots of eggs from the original batch with the exception of the first three DNA phosphorus determinations of Experiment 1. The results are shown in Figure 1.

The typical increase in the rate of respiration is indicated by the upper two curves. Quantitatively the uppermost of these is very similar to that published by Horowitz (1940) for this species. The curves for the amount of DNA phosphorus in the embryos parallel those of the rate of respiration except for the initial hour or two during which the latter increase rapidly and the former do not. The final

plateau of the curves of Experiment 1 may be the result of starvation; the larvae do not develop further than the short-armed pluteus stage without feeding.

These data indicate that in both experiments the respiration curves parallel the corresponding curves for DNA phosphorus. To help determine whether any relationship exists between the two phenomena, correlation coefficients were calculated. The coefficient of correlation, r, for the combined data of both experiments, covering the developmental period from the 2-cell stage to the pluteus, with fourteen paired values of rates of oxygen consumption and amounts of DNA phosphorus, is 0.936. This excludes the values for the unfertilized egg, which is assumed to be very different physiologically from the developing egg. The correlation coefficient

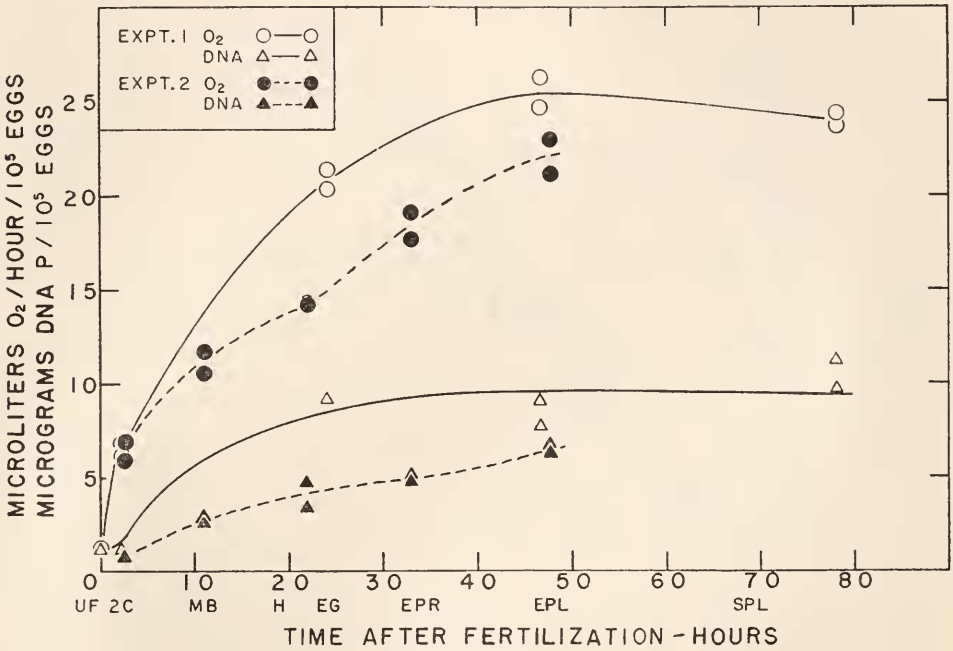

FIGURE 1. Rate of oxygen consumption and DNA phosphorus content in the embryos of *Strongylocentrotus purpuratus*. Developmental stages: UF, unfertilized; 2C, two-cell; MB, middle blastula; H, hatching; EG, early gastrula; EPR, early prism; EPL, early pluteus; SPL, short-armed pluteus.

for the second experiment alone, for which the data are more complete with ten paired values, is 0.978. These correlation coefficients are much higher than necessary to be significantly different from zero at the 1% level. A scatter diagram with the regression lines for the two experiments is shown in Figure 2.

In the unfertilized egg the rate of oxygen consumption, from the data of the first experiment, is 1.24 microliters per hour per egg while the rate of 8.34 microliters per hour per egg is the value predicted from the regression line $y = 2.04x + 6.05$ knowing that the amount of DNA phosphorus is 1.12 micrograms in the unfertilized egg. The observed value is not quite significantly different from the calculated one at the

5% level.[3] Unfortunately the amount of DNA phosphorus and the rate of oxygen consumption previous to fertilization for the eggs used in Experiment 2, for which the standard error around the regression line is much less than that for the combined experiments, were not determined so that the test for significance cannot be employed using only this second experiment. However, the data for respiration and DNA phosphorus in the unfertilized egg have been confirmed many times in other experiments by ourselves and other workers using the methods employed here, and it is quite likely that a significant difference between the respiration in the unfertilized egg and the amount expected from the regression line would be found. It may be

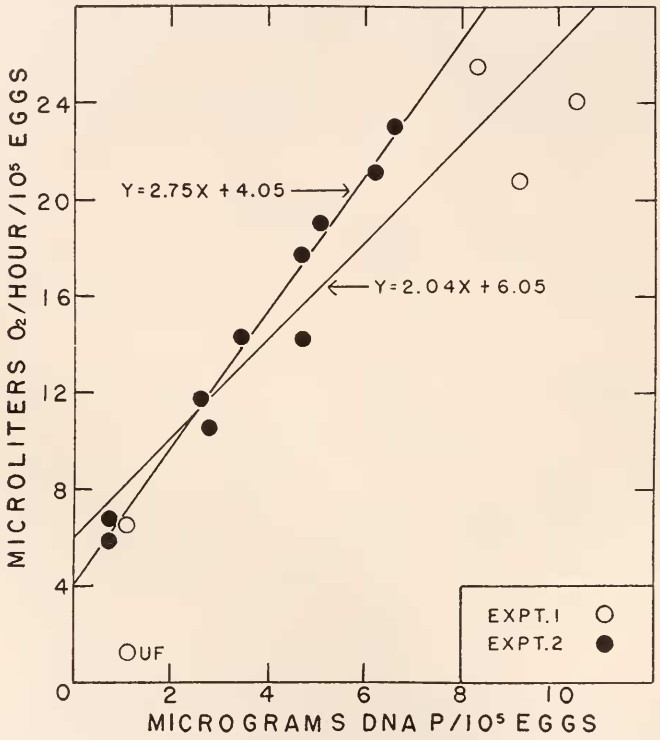

FIGURE 2. Scatter diagram with regression lines for the rate of oxygen consumption, y, and amount of DNA phosphorus, x, in developing embryos. The equation of the line for Experiment 2 is $y = 2.75x + 4.05$ and for the combined experiments is $y = 2.04x + 6.05$.

tentatively concluded that in the unfertilized egg respiration is not related to DNA in the same manner as it is in the developing embryo.

The curves obtained in Experiment 1 (Fig. 1), both for the rate of respiration and DNA synthesis, are higher than those of Experiment 2. Experiment 1 was done in February during the height of the breeding season whereas Experiment 2

[3] The t test was used for this determination. A value of 2.18 for t is necessary for significance at the 5% level for 12 degrees of freedom, and the calculated value was -2.12; therefore, the probability is approximately 0.06.

was done in August, so that the dissimilarity could be due to a difference in winter and summer eggs. It was found by Fox (1938) that at a given temperature of about 20° C. for *Psammechinus* and *Paracentrotus*, material from colder waters cleaved more rapidly than material from warmer waters. In an analogous way, it may be that the rate of oxygen consumption and the synthesis of DNA would be greater for winter eggs than summer eggs when measured at the same temperature. Also, in each experiment all of the analyses were made on the eggs of one sea urchin so that the difference in the curves may be due to individual variation. The differences obtained cannot be accounted for by the variation in the egg counts.

Discussion

It is most probable that there is a relationship between the rate of oxygen consumption and DNA synthesis beginning with the 2-cell stage and extending into the pluteus stage. It is possible that the two phenomena are related only indirectly through other aspects of the metabolism; such indirect relationships could be very complicated and would be fruitless to consider without further information. If the relationship is direct, there are two possibilities, the first of which is that such a correlation exists because of the use of oxygen to produce energy to synthesize DNA. This relationship was indicated by Brachet (1938) on the basis of his experiments with *Chaetopterus* eggs, which were cited earlier. However, aerobic energy is used for many purposes and it is rather unlikely that a limitation of the synthesis of DNA by aerobic energy would be observable in the over-all rate of oxygen consumption. The second possibility for a direct relationship is more likely; it is that the correlation exists because of the control of the rate of oxygen consumption by the amount of DNA. The rate of respiration could be controlled by the amount of DNA if the latter were limiting the synthesis or activation of enzymes. It is known that chromosomes are largely composed of desoxyribonucleoprotein which is thought to be associated with the genetically active part of the chromosomes, the genes. Current theories of gene action propose that the primary effect of genes is to confer the final specificity on enzymes, usually one gene controlling one enzyme. However, these theories are concerned with the kind of genes and enzymes whereas the correlation discussed above is concerned with quantities. It would not be merely the number of genes present at any stage of development that dictates the activity of the respiratory enzymes, because, as was pointed out earlier, the number of cells, and, therefore, the number of genes, bears no relation to the respiration. It must be concluded, if a cause and effect relation is found to exist here, that the amount of respiratory activity is dependent on the total amount of DNA present in the embryo.

It was tentatively concluded that the respiration of the unfertilized egg is lower than expected judging from the amount of DNA phosphorus present. It is possible that before fertilization the activity of enzymes previously stored in the eggs is suppressed and respiration is low. After fertilization these enzyme systems could function at a greater capacity possibly due to a protoplasmic rearrangement (Brachet, 1950). This rearrangement is reflected in the increased rate of oxygen consumption immediately following fertilization, which, therefore, does not need to involve synthesis of DNA or new enzymes. After the utilization to full capacity of these stored enzymes, the synthesis or activation of more enzymes would be dependent on the

synthesis of DNA, and from then on, the rising rate of oxygen consumption would parallel the increasing amount of DNA.

The values obtained for DNA phosphorus in this investigation approximate those obtained by Schmidt, Hecht and Thannhauser (1948) and Villee, Lowens, Gordon, Leonard and Rich (1949). Both groups of workers analyzed the eggs of *Arbacia punctulata* by the method of Schmidt and Thannhauser. Schmidt and coworkers found that the DNA phosphorus per embryo varied between 0.6 and 0.9 $\times 10^{-5}$ micrograms in the unfertilized egg and increased to 10.7 $\times 10^{-5}$ micrograms in the 24-hour pluteus. Villee and co-workers found an increase from 1.2 $\times 10^{-5}$ to 7.0 $\times 10^{-5}$ micrograms of DNA phosphorus per embryo from the third to the twentieth hour of development. The comparable results of the present study are 1.1 $\times 10^{-5}$ micrograms of DNA phosphorus per embryo in the unfertilized egg and 10.4 $\times 10^{-5}$ micrograms in the pluteus.

Summary

1. Following an abrupt rise in rate of respiration of the egg of the sea urchin at the time of activation there is a period of continued increase extending into the pluteus larval stage. This is not explainable by means of the increase in cell number, cleavage rate, or nuclear volume.

2. In the developing embryo of *Strongylocentrotus purpuratus* (Stimpson) the increase in the rate of oxygen consumption has been found to have a very high positive correlation with the amount of desoxyribonucleic acid (DNA) phosphorus present in the embryo. The correlation coefficient was found to be much higher than necessary to be significantly different from zero at the 1% level. The regression coefficient for the linear regression of the rate of respiration on the amount of DNA phosphorus for the best studied case was found to be 2.75 microliters of oxygen per microgram of DNA phosphorus per hour.

3. The same correlation probably does not exist in the unfertilized egg.

4. If the relation is a direct one, the most reasonable interpretation of the correlation found is that the synthesis or activation of respiratory enzymes is under nucleic acid control.

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